REMARKS

I. Restriction Requirement and Claim Amendment

Claim 1 is amended. Claims 2 and 3 are canceled. Support for the amendment to Claim 1 is found on page 2, line 19 and pages 17, line 19 of the instant application.

The Examiner issued a restriction requirement, requiring Applicants to elect one of the following four groups of claims for further prosecution:

I. Claims 1-24 (methods of treating a condition mediated by a proinflammatory cytokine);

II. Claims 25-34 (methods of determining whether a compound is an agonist selective for an α7 nicotinic receptor);

III. Claims 35-48 (methods of determining whether a compound is an antagonist reactive with an α7 nicotinic receptor); and

IV. Claims 49-55 (oligonucleotides and mimetics inhibiting attenuation of TNF release).

The Examiner also required the Applicants to elect a single disclosed group of compounds within any of the following five subgroups:

1) spiroazabicyclic compounds;

2) spiroazabicyclic heterocyclic compounds;

3) anabaseine derivatives;

4) tropanes; and

5) quaternary analogs of cocaine.

The Examiner also requested that the Applicants elect one class of disease from class A through T as well as elect a single species from the elected group:

A. Inflammatory conditions;

B. Diseases caused by a viral agent;

C. Diseases caused by agent other than virus;

D. Immunoregulatory diseases;

E. Achalasia;

F. Cardiovascular disorders;

G. Organ necrosis;

H. Cachexia;

I. Heperpyrexia;

J. Cancerous disorders selected from eosiniphilic granuloma, granulomatosis and sarcoidosis;

K. Pulmonary and respiratory conditions;

L. Hydratid cysts;

M. Burns;

N. Atherosclerosis;

O. Coeliac disease;

P. Spinal chord injury and paralysis;

Q. Arthralgias;

R. Paget's disease; and

T. Hodkin's disease.

It is Applicants' understanding that upon allowance of a generic claim, Applicant will be entitled to consideration of claims drawn to additional species.

A provisional election of Group I, subgroup 3, diseases of Class A with election of rheumatoid arthritis was made by Steven G. Davis, Esq. on April 28, 2005 during a telephonic conversation with Examiner M. Graffeo. Applicants affirm the provisional election and further clarify the election of species for the purposes of searching.

Applicants hereby confirm the election of the invention of Group I, Claims 1-24 drawn to methods of treating a condition mediated by a proinflamatory cytokine.

Within claims of Group I, Applicants confirm the election of chemical genera of subgroup 3 (anabaseine derivatives). Claims readable on the elected genera are Claims 1-4, 10-15 and 20-24.

Within claims of Group I, subgroup 3, Applicants confirm the election of diseases of class A (inflammatory conditions). Claims readable on the elected species are Claims 1-4, 10-15 and 20-23. Applicants further confirm the election of the species of rheumatoid arthritis for the purposes of searching.

II. Applicants Invention

Scientific background will be given with reference to Exhibit A, "MBC 3320 Acetylcholine", downloaded from the URL:

http://www.neurosci.pharm.utoledo.edu/MBC3320/acetylcholine.htm

on October 10, 2005.

Cholinergic receptors can be divided into two types, muscarinic and nicotinic, based on pharmacological action of various agonists and antagonists. Nicotinic receptors produce pharmacologically and physiologically distinct responses from muscarinic receptors although the same neurotransmitter, acetylcholine, stimulates both types of cholinergic receptors. (Exhibit A, page 1, 3rd paragraph.)

Nicotinic receptors are pentamers. (Exhibit A, page 2, 2nd paragraph.) There are five classes of subunits: α, β, γ, δ, and ε. Within each class of subunit, multiple types exist. Thus, there are nine known α-subunit types, four known β -subunit types, etc. The subunit composition of nicotinic receptors varies among tissue types (Exhibit A, page 2, last paragraph). α-Bungarotoxin binds to various types of both α and β subunits (Exhibit A, page 2, 3rd paragraph and page 3, the Table). Accordingly, without more one cannot tell which subunits make up an α-bungarotoxin-sensitive receptor.

Applicants have discovered that activation of a ***specific type of nicotinic receptors***, the α7 cholinergic receptor, can inhibit an inflammatory response and therefore has utility in treating a wide range of inflammatory disorders including rheumatoid arthritis. This role for α7 receptor was heretofore unknown in the art. This discovery enabled Applicants to develop therapeutically effective methods of treating patients with inflammatory disorders by utilizing α7-specific agonists without eliciting effects resulting from activation of other cholinergic receptors,. Furthermore, because the role of α7 cholinergic receptors in attenuating inflammatory responses, including those involved in rheumatoid arthritis, was unknown until the Applicants' discovery, there was, correspondingly, no teaching or suggestion of selecting a cholinergic agonist selective for an α7 nicotinic receptor for treating a patient suffering from an inflammatory disorder, as recited in Claim 1 as amended.

Accordingly, the results presented in the present application are unexpected and surprising. Specifically, the data presented in Examples 3 - 6 demonstrates that anabaseine

compounds (V) and (VI) are effective in inhibiting TNF-α release by virtue of their α7-specific agonist activity.

In addition to demonstrating unexpected results, Applicants' invention offers advantages over prior methods of treating patients suffering from condition mediated by release of a proinflammatory cytokine. Namely, it is expected that pharmaceutical compositions comprising α7 receptor-subunit-selective agonists would be likely to have fewer side-effects than agonists that non-selectively activate cholinergic receptors. See page 4, lines 27-30 of the instant application.

III. Claim Rejection under 35 U.S.C. §103(a)

Claims 1-4, 10-15 and 20-23 are rejected under 35 U.S.C. §103(a) as being unpatentable over Borovikova *et al.*, "Vagus Nerve Stimulation Attenuates the Systemic Inflammatory Response to Endotoxin", Let. to Nature (2000) 405:458-462, in view of Moreland *at al.*, "Treatment of Rheuamtoid Arthritis with Recombinant Human Tumor Necrosis Factor (p75) - Fc Fusion Protein", New England J. Med. (1997) 337:141-147, and further in view of U.S. Pat. No.5,977,144 to Meyer *et al.*

The Examiner stated that Borovikova *et al.* teach that α-bungarotoxin-sensitive nicotinic acetylcholine receptors are required for inhibition of TNF-mediated response and that Moreland *et al.* teach that TNF plays a role in the pathogenesis of rheumatoid arthritis. The Examiner further stated that Meyer *et al.* teach that α7 nicotinic receptors are, in fact, α-bungarotoxin-sensitive and also teach that specific anabaseine derivatives are selective for α7 receptors. The Examiner further stated that one skilled in the art looking to agonize the α7 nicotinic receptors would be motivated to look to popular and known α7-specific agonists such as those disclosed in Meyer *et al.*

Applicants respectfully disagree with the Examiner's analysis of motivation to combine the cited art.

As Applicants explained above in section II of the instant reply, the instant invention is based on the discovery that from among the general class of α-bungarotoxin-sensitive receptors, it is the α7 cholinergic receptor that attenuates inflammatory responses in a number of conditions. The combined art of Borovikova *et al.* and Moreland *et al.* fails to identify the α7

cholinergic receptor as the relevant receptor subtype from among α-bungarotoxin-sensitive receptors generally, and therefore fails to motivate one skilled in the art to select α7-specific agonists. Meyer *et al.* do not remedy this deficiency of Borovikova *et al* and Moreland *et al.* Although Meyer *et al.* discloses certain α7-specific agonists, nothing in Meyer *et al.* teaches or suggests that the α7-specific agonists disclosed therein can be used for modulating inflammatory responses. Because multiple classes of nicotinic acetylcholine receptors are α-bungarotoxin-sensitive, mere disclosure in Meyer *et al.* that α7 nicotinic receptors are, in fact, α-bungarotixin-sensitive is insufficient to motivate one skilled in the art to select α7-specific agonists without knowledge that activation of α7 cholinergic receptors can alleviate inflammatory response.

Applicants further submit that absent evidence in the cited art of a connection between the α7 cholinergic receptors and the alleviation of disorders recited in Claim 1 of the instant application, the Examiner's argument amounts to the impermissible "obvious to try" rationale. (M.P.E.P. §2145.X.B.)

Indeed, multiple classes of nicotinic acetylcholine receptors are α-bungarotoxin-sensitive. Even though Meyer *et al.* teach one such class, a receptor that includes α subunit of type 7, Meyer *et al.* do not make a connection between the specific type of the receptors and the ability to inhibit inflammatory response, as recited in Claim 1 of the instant application. The combined teachings of Borovikova *et al.* and Moreland *et al.* do not help one skilled in the art to make this connection as neither reference teaches that the responsible subunit is the α7 subunit. Thus, the Examiner's conclusion that the cited reference are properly combinable amount to a suggestion, in the words of M.P.E.P. §2145 "to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful results". Applicants submit that the combination of the cited references is improper as an application of the "obvious to try" rationale.

Reconsideration and withdrawal of the rejection are respectfully requested.

IV. Double Patenting Rejections

(1) *Rejection of Claims 1-4, 10-15 and 20-23 Under Obviousness-type Double Patenting Doctrine over Claims 1-8, 11-14 and 18 of U.S. Pat. No. 6,838,471*

Claims 1-4, 10-15 and 20-23 are rejected Under Obviousness-type Double Patenting Doctrine over Claims 1-8, 11-14 and 18 of U.S. Pat. No. 6,838,471 to Tracey (hereinafter, "Tracey I") in view of U.S. Pat. No. 5,977,144 to Meyer *et al.*

It is the Applicants' understanding that the Examiner reached the stated conclusion based on arguments essentially identical to the arguments made in advancing the rejection under 35 U.S.C. §103: the referenced claims of Tracey I teach a method of inhibiting the release of pro-inflammatory cytokines from cells comprising treating said cell with an agonist selective for α-bungarotoxin-sensitive nicotinic acetylcholine receptor; and Meyer *et al.* teach that α7 nicotinic receptors are, in fact, α-bungarotoxin-sensitive and also teach that specific anabaseine derivatives are selective for α7 receptors.

Applicants respectfully disagree with the Examiner's analysis of motivation to combine the cited art.

Similarly to the art relied upon by the Examiner in advancing the rejection under 35 U.S.C. §103(a), nothing in the referenced claims of Tracey I teaches or suggests that from among α-bungarotoxin-sensitive receptors generally, it is the α7 nicotinic receptor subtype that can inhibit inflammatory responses, as recited in Claim 1 of the instant application. There are multiple classes of nicotinic acetylcholine receptors that are α-bungarotoxin-sensitive and the referenced claims of Tracey I do not direct one skilled in the art to seek agonists specific for the α7 subtype. As presented above by Applicants, because multiple classes of nicotinic acetylcholine receptors are α-bungarotoxin-sensitive, mere disclosure in Meyer *et al.* that α7 nicotinic receptors are, in fact, α-bungarotoxin-sensitive is insufficient to motivate one skilled in the art to select α7-specific agonists without knowledge that activation of α7 cholinergic receptors can alleviate inflammatory response.

Furthermore, as presented above by Applicants, absent evidence in the cited art of a connection between the α7 cholinergic receptors and the ability to inhibit inflammatory responses, the Examiner's argument amounts to the impermissible "obvious to try" rationale. (M.P.E.P. §2145.X.B.)

Reconsideration and withdrawal of the rejection are respectfully requested.

(2) *Rejection of Claims 1-4, 10-15 and 20-23 Under Obviousness-type Double Patenting Doctrine over Claims 1-3, 5-6, 14 and 16 of U.S. Pat. No. 6,610,713*

Claims 1-4, 10-15 and 20-23 are rejected Under Obviousness-type Double Patenting Doctrine over Claims 1-3, 5-6, 14 and 16 of U.S. Pat. No. 6,610,713 to Tracey (hereinafter, "Tracey II") in view of U.S. Pat. No. 5,977,144 to Meyer *et al.*

It is the Applicants' understanding that the Examiner reached the stated conclusion based on arguments essentially identical to the arguments made in advancing the rejection under 35 U.S.C. §103 and the double patenting rejection over Tracey I in view of Meyer *et al.* Namely, the referenced claims of Tracey II teach a method of inhibiting the release of pro-inflammatory cytokines from cells comprising treating said cell with ***a*** cholinergic agonist; Meyer *et al.* teach that specifc anabaseine derivatives are selective for α7 receptors, ***a*** class of nicotinic acetylcholine receptors which in turn are a class of cholinergic receptors.

Applicants respectfully disagree with the Examiner's analysis of motivation to combine the cited art.

As described above, cholinergic receptors encompass both muscarinic receptors and nicotinic acetylcholine receptors. As Applicants explained in section II of the instant reply, nicotinic acetylcholine receptors can comprise five classes of subunits: α, β, γ, δ, and ε. Within each class of subunit, multiple types exist. For example, there are nine α-subunit types, four β - subunit types, etc. Nothing in the referenced claims of Tracey II, which are directed to the administration of a cholinergic agonist, directs one skilled in the art toward an agonist selective for α7 receptors. Meyer *et al.* fails to teach or suggest that activation of the α-7 receptors by the compounds disclosed by Meyer *et al.* treats or alleviates disorders recited either in the referenced claims of Tracey II or in Claim 1 of the present application. Because multiple classes of nicotinic acetylcholine receptors are α-bungarotoxin-sensitive, mere disclosure in Meyer *et al.* that α7 nicotinic receptors are, in fact, α- bungarotoxin-sensitive is insufficient to motivate one skilled in the art to select α7-specific agonists without knowledge that activation of α7 cholinergic receptors can alleviate an inflammatory response.

Applicants submit, therefore, that neither Tracey II nor Meyer *et al.* would motivate one skilled in the art to select a species-specific agonist (such as an agonist selective for an α7

cholinergic receptor required by the instant Claim 1) without knowledge that activation of this species of acetylcholine receptors can alleviate inflammatory responses.

Absent evidence in the cited art of a connection between the α7 cholinergic receptors and the disorders recited in Claim 1 of the instant application, the Examiner's argument amounts to the impermissible "obvious to try" rationale. (M.P.E.P. §2145.X.B.) Such evidence is clearly absent in Tracey II and Meyer *et al.*

Applicants respectfully disagree with the Examiner's analysis of motivation to combine the cited art.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By /Alexander Akhiezer/
Alexander Akhiezer
Registration No. 54,617
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

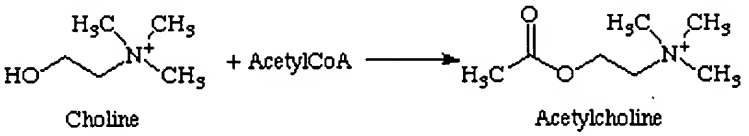
Concord, MA 01742-9133
Dated: 11/4/05

MBC 3320 Acetylcholine

Nicotinic receptors Muscarinic receptors Alzheimer's disease Acetylcholinesterase

This page was last updated on January 20, 2000 at 2:00 p.m.

The first neurotransmitter system to be covered will be the cholinergic system. Acetylcholine was one of the first neurotransmitters to be discovered, (originally called "vagusschtuff" because it was found to be the substance released by stimulation of the vagus nerve that altered heart muscle contractions).



Choline Acetylcholine

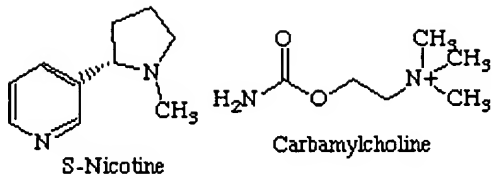
Acetylcholine is produced by the synthetic enzyme choline acetyltransferase which uses acetyl coenzyme A and choline as substrates for the formation of acetylcholine. Dietary choline and phosphatidylcholine serve as the sources of free choline for acetylcholine synthesis. Upon release, acetylcholine is metabolized into choline and acetate by acetylcholinesterase, and other nonspecific esterases. Acetylcholine release can be excitatory or inhibitory depending on the type of tissue and the nature of the receptor with which it interacts.

Cholinergic receptors can be divided into two types, muscarinic and nicotinic, based on the pharmacological action of various agonists and antagonists. Muscarinic receptors originally were distinguished from nicotinic receptors by the selectivity of the agonists muscarine and nicotine respectively. Muscarinic receptors will be discussed in detail later, while nicotinic receptors will be discussed in the next section.

Nicotinic cholinergic receptors

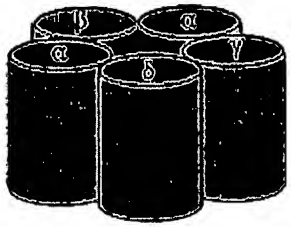
Nicotinic receptors produce pharmacologically and physiologically distinct responses from muscarinic receptors, although acetylcholine (and other agonists such as carbamylcholine) stimulates each type of response. Nicotinic responses are of fast onset, short duration and excitatory in nature. The pharmacology of nicotinic receptors has been studied in great detail and our understanding of how ion channel-coupled neurotransmitter receptors work is based largely on the study of this class of proteins.

Nicotinic receptors are found in a variety of tissues, including the autonomic nervous system, the neuromuscular junction and the brain in vertebrates. They also are found in high quantities in the electric organs of various electric eels and rays. The high quantities of receptors in these tissues and the use of neurotoxins from snake venom (e.g., cobra venom) that bind specifically to the nicotinic receptor aided the purification of the receptor protein (see below).



Agonists such as acetylcholine, carbamylcholine and nicotine produce the physiological responses associated with nicotinic cholinergic activation. Acetylcholine produces an influx of sodium through a ligand-gated ion channel. Acetylcholine and carbamylcholine also stimulate muscarinic receptors and therefore should be considered mixed cholinergic agonists.

The amino acid sequence for the nicotinic receptor was determined after solubilization of the receptor from the electric organ of *Torpedo californica* using anionic detergents such as sodium dodecyl sulfate, passing the receptor through an affinity column containing α bungarotoxin (from snake venom) and washing the receptor from the column. Subsequently, molecular biological techniques were used to clone additional receptor subunits. The nicotinic receptor consists of five polypeptide subunits. The amino acid sequence for the α subunits consists of a glycolipid region (which contains the ACh binding site and a sulfhydryl groups) with four hydrophobic regions that span the membrane. Nine α subunits have been cloned, along with four β subunits. In the neuromuscular junction, δ and γ subunits also have been identified. The γ subunit is replaced by an ε subunit in the adult muscle.



α-Bungarotoxin binds to the α and β subunits and probably blocks both the channel and the ACh binding site. Local anesthetics and other compounds such as phencyclidine bind to the receptor, apparantly at the site of the sodium channel and modulate the binding of acetylcholine to the active site. Local anesthetics also prevent ion conductance through a direct action at the channel. The sodium channel and the channel for the nicotinic cholinergic receptor have some similar properties (in both structure and sensitivity to drug action) and may have a common genetic origin.

In general terms, acetylcholine binds to the α-subunits of the receptor making the membrane more permeable to cations and causing a local depolarization. The local depolarization spreads to an action potential or leads to muscle contraction when summed with the action of other receptors. Nicotinic receptors possess a relatively low affinity for acetylcholine at rest. The affinity for acetylcholine is increased during activation (through an allosteric mechanism which increases the likelihood of another molecule of acetylcholine binding to the other α subunit). At high concentrations of acetylcholine, the affinity for acetylcholine becomes higher and the receptor subsequently becomes desensitized. The ionophore (ion channel) is open during the active state and local anesthetics may bind to the open channel.

The subunit composition of nicotinic receptors differs in skeletal muscle, autonomic ganglia and brain. The table below lists some of the properties of receptors found in different tissues. Note that multiple subunit compositions are possible, which may permit the development of compounds selective for a

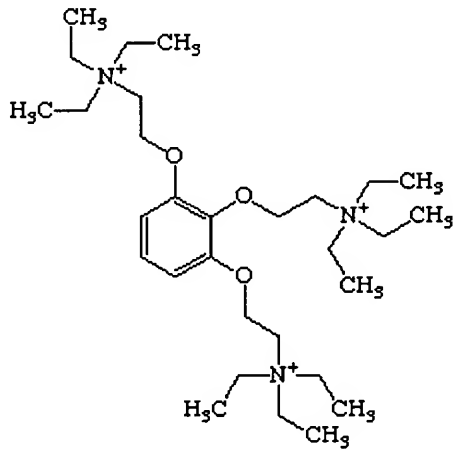
particular combination. Within the CNS, the α4β2 combination predominates.

Nicotinic Acetylcholine Receptors

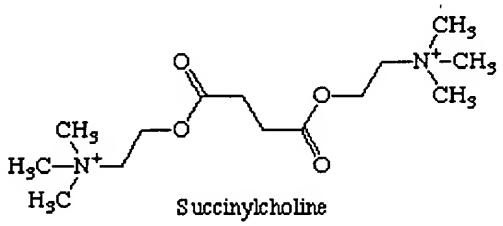
Receptor	Skeletal muscle	Autonomic ganglion	CNS	CNS
Subunits	α1,β1,δ,γ(ε)	α3,α5,α7,β2,β4	α3,α4,β2,β4	α7,α8,α9
α-Bungarotoxin	+	+/-	-	+
Antagonists	α-Bungarotoxin	Hexamethonium	Dihydro-β-erythroidine Mecamylamine	α-Bungarotoxin Mecamylamine
Agonists	Epibatidine	Epibatidine	Epibatidine ABT-418	

Nicotinic antagonists

Antagonists for nicotinic receptors include such diverse compounds as curare, α-bungarotoxin and gallamine. Nicotinic receptors found at the neuromuscular junction differ from the receptors found in autonomic ganglia and can be distinguished both pharmacologically and biochemically.

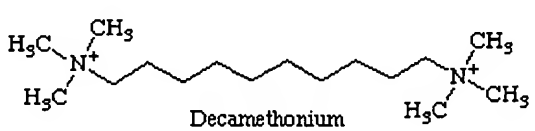


Gallamine

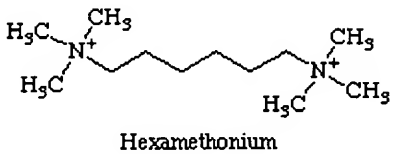


Succinylcholine

Gallamine (a mixed muscarinic and nicotinic antagonist) and decamethonium are more effective antagonists at the neuromuscular junction than at the autonomic ganglia. The spacing of the charged nitrogens seems to be of critical importance in the selectivity of the drugs. Gallamine and succinylcholine are used during surgery to block nmj receptors and produce paralysis. Succinylcholine is used more often because it can be metabolized by acetylcholinesterase to produce inactive compounds. Note the structural similarity to acetylcholine. Decamethonium is another nicotinic antagonist with some selectivity for the neuromuscular junction



Decamethonium



Hexamethonium

Ganglionic blockers include the quaternary compounds hexamethonium and tetraethylammonium as

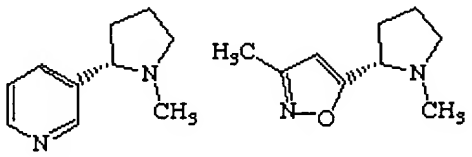
well as the tertiary and secondary amines mecamylamine and pempidine. While quaternary amines competitively inhibit cholinergic responses in autonomic ganglia, tertiary and secondary amines also have a noncompetitive component.

Ganglionic blockers are used to treat hypertension in some cases. Because they block both sympathetic and parasympathetic responses, their use is restricted to emergency situations or circumstances where the patient can be monitored (orthostatic hypotension is one of the common side effects).

Succinylcholine and decamethonium are both depolarizing blockers of nicotinic receptors, in that they initially mimic the action of acetylcholine. Following the initial depolarization, the depolarizing blockers exert a long-acting blockade of the receptor, thereby preventing further activation by acetylcholine. The trimethylammonium group seems to be important for action as a depolarizing blocker since compounds with a triethylammonium group do not cause the depolarization but do block the action of acetylcholine (see gallamine for instance).

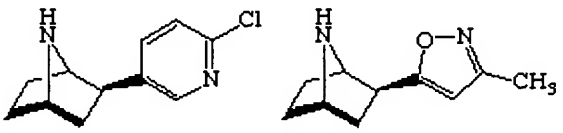
Nicotinic agonists

Over the past several years, a variety of research groups have focused on the development of selective nicotinic agonists. Nicotinic agonists could be useful in the treatment of a variety of neurological disorders including Alzheimer's disease, Parkinson's disease and chronic pain. Epibatidine is a nicotinic agonist isolated from the skin of an Ecuadoran frog *Epipedobates tricolor* that displays potent analgesic properties.



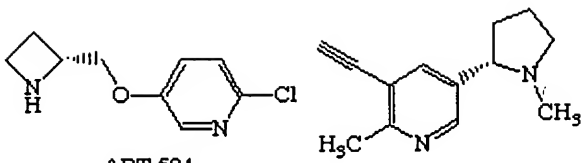
S-Nicotine ABT-418

Another nicotinic agonist, ABT-418, exhibits some cognition enhancing properties. Note its similarity to nicotine, with an ixoxazole moiety replacing the pyridyl group of nicotine. Epiboxidine is a structural analogue that combines elements of both epibatidine and ABT-418. It also is a potent nicotinic agonist.



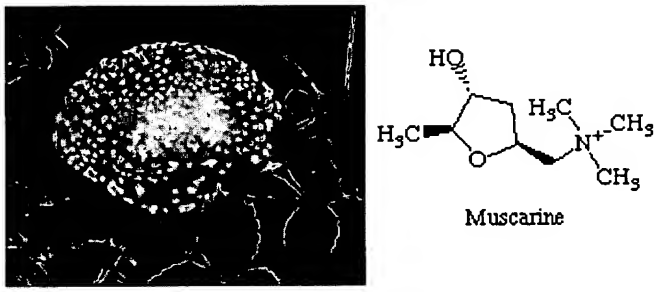
Epibatidine Epiboxidine

Two other derivatives are worth noting. The azetidine analogue of epibatidine, ABT-594, is a potent analgesic with significantly fewer side effects than epibatidine. SIB-1508 is another nicotinic agonist with potential utility in the treatment of Parkinson's disease.



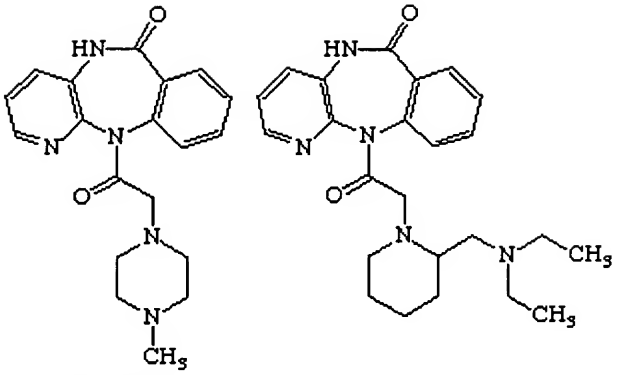
Muscarinic receptors

Acetylcholine and carbamylcholine can bind to both muscarinic and nicotinic receptors, yet the responses elicited by activating each receptor differ in several ways. Muscarinic responses are slower, may produce excitation or inhibition and involve second messenger systems, rather than the direct opening of an ion channel. Muscarinic receptors are G protein-coupled receptors and mediate their responses by activating a cascade of intracellular pathways. Muscarine is the prototypical muscarinic agonist and derives from the fly agaric mushroom *Amanita muscaria*. Like acetylcholine, muscarine contains a quaternary nitrogen important for action at the anionic site of the receptor (an aspartate residue in transmembrane domain III). Most muscarinic agonists obey the "rule of five" atoms from the quateranry ammonium moiety to the terminal atom.



Muscarinic receptors are found in the parasympathetic nervous system. Muscarinic receptors in smooth muscle regulate cardiac contractions, gut motility and bronchial constriction. Muscarinic receptors in exocrine glands stimulate gastric acid secretion, salivation and lacrimation. Muscarinic receptors also are found in the superior cervical ganglion where they can produce at least two physiologically distinct responses. In addition, muscarinic receptors are found throughout the brain, including the cerebral cortex, the striatum, the hippocampus, thalamus and brainstem.

In general the classical muscarinic antagonists such as atropine recognize a single class of binding sites as determined in binding assays. In the 1980's, several selective muscarinic antagonists were identified. Pirenzepine was very useful in the characterization of M_1 muscarinic receptors, while AF-DX 116 was used to identify M_2 receptors in the heart. M_3 receptors are found in smooth muscle and in both exocrine glands (e.g., lacrimal glands) and endocrine glands (e.g., pancreas). Muscarinic agonists bind heterogeneously to receptors in both the brain and peripheral nervous system.



Pirenzepine AF-DX 116

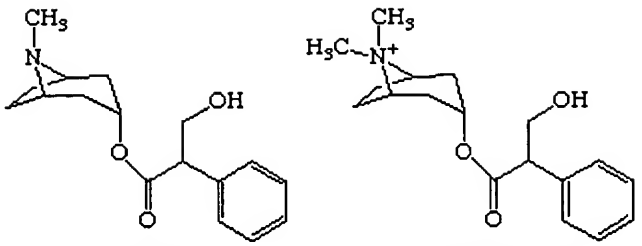
In the late 1980's, molecular cloning techniques identified five different subtypes of muscarinic receptors. Each receptor shares common features including specificity of binding for the agonists acetylcholine and carbamylcholine and the classical antagonists atropine and quinuclidinyl benzilate. Each receptor subtype couples to a second messenger system through an intervening G-protein. M_1, M_3 and M_5 receptors stimulate phosphoinositide metabolism while M_2 and M_4 receptors inhibit adenylate cyclase. The tissue distribution differs for each subtype. M_1 receptors are found in the forebrain, especially in the hippocampus and cerebral cortex. M_2 receptors are found in the heart and brainstem while M_3 receptors are found in smooth muscle, exocrine glands and the cerebral cortex. M_4 receptors are found in the neostriatum and M_5 receptor mRNA is found in the substantia nigra, usggesting that M_5 receptors may regulate dopamine release at terminals within the striatum. The structural requirements for activation of each subtype remain to be elucidated.

Muscarinic Acetylcholine Receptors

	M_1	M_2	M_3	M_4	M_5
Distribution	Cortex, hippocampus	Heart	Exocrine glands, GI tract	Neostriatum	Substantia nigra
Antagonists	Pirenzepine	AF-DX 116	pF-HHSiD		
Agonists	Xanomeline, CDD-0097				
G protein	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$
Intracellular response	Phospholipase Cβ	Adenylyl cyclase inhibition	Phospholipase Cβ	Adenylyl cyclase inhibition	Phospholipase Cβ

Muscarinic antagonists

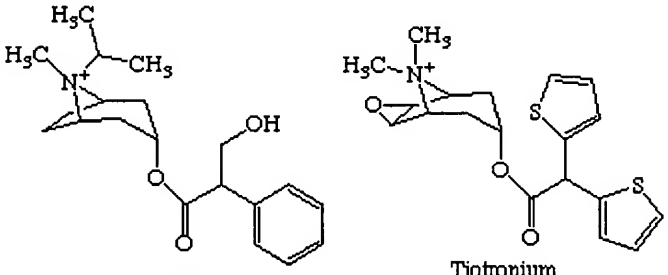
Muscarinic antagonists such as scopolamine and atropine are among the oldest known molecules, originally derived from natural sources. They are both alkaloids (natural, nitrogenous organic bases, usually containing tertiary amines) from the nightshade plant *Atropa belladonna*. The presence of an N-methyl group on atropine or scopolamine changes the activity of the ligand, possibly by preventing a close interaction between the ligand and the membrane or lipophilic sites on the receptor. The methyl group also prevents the penetration into the brain.



Atropine Methyl-atropine

The potent anticholinergics are used to control the secretion of saliva and gastric acid, slow gut motility, and prevent vomiting. They also have a limited therapeutic use for the treatment of Parkinson's disease. In large doses however, the muscarinic antagonists with tertiary amines have severe central effects, including hallucinations and memory disturbances.

In recent years, the quaternary muscarinic antagonist ipratroprium has been used in the treatment of chronically obstructed pulmonary disorder as an adjunct to β_2 agonist therapy. M_3 muscarinic receptors mediate bronchoconstriction in the airways. Muscarinic antagonists such as ipratropium and the long-lasting tiotropium are effective bronchodilators.



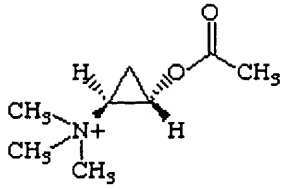
Ipratropium Tiotropium

The possible use of presynaptic antagonists to increase acetylcholine levels has attracted some attention recently. Muscarinic autoreceptors resemble pharmacologically the M_2 receptor found in the heart. M_2 antagonists enhance acetylcholine release by blocking the feedback inhibition produced by the action of acetylcholine on presynaptic terminals.

Muscarinic agonists

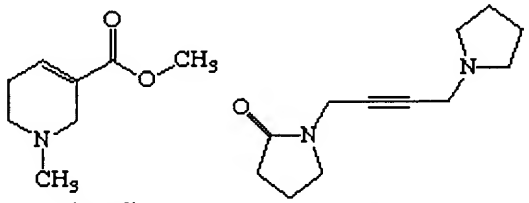
The ability for the quaternary ammonium group to fit into an anionic site on muscarinic receptors may be an important factor for the binding of a ligand to muscarinic receptors. For an example of the requirement of the quaternary amine moiety, condsider that dimethylaminoethylacetate (the tertiary form of acetylcholine) is 1000-fold less than acetylcholine, in part due to a lower affinity for the receptor.

The molecule of acetylcholine is flexible and may form an infinite number of conformations from the extended to the quasi-ring structure. The three-membered ring of acetoxycyclopropyl-trimethylammonium iodide demonstrates the concept that the extended form of acetylcholine contains the highest intrinsic activity. The trans isomer has much higher activity than the cis isomer which orients the ester and the quaternary amine together.



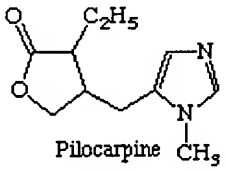
Trans-acetoxycyclopropyl-trimethylammoinium

While the quaterany nitrogen is essential for eliciting full muscarinic responses with muscarinic agonists, there are a few potent muscarinic agents which contain tertiary amines (e.g., arecoline, oxotremorine and pilocarpine). They are potent both peripherally and centrally although they are of limited therapeutic value because of the wide range of cholinergic responses that they elicit. Oxotremorine is of interest because of its ability to produce tremors, thereby providing an early model for Parkinson's disease.



Arecoline Oxotremorine

Simple tertiary amines do not show considerable potency for the receptor, but this can be counteracted if the rest of the molecule binds potently to the receptor (e.g., through an ester bioisostere). Oxotremorine fills this role with an amide group in a pyrrolidone ring as the nitrogen replaces oxygen in a hydrogen bond acceptor role. Arecoline (isolated originally from the betel nut) has a reversed ester acetylcholine profile, while pilocarpine has its ester in the cyclic form of a lactam ring, which may help increase the binding interaction. In general, it is important to have two sites for hydrogen bond acceptance in the ester isostere. The orientation of the ester isostere may be important for selective action as well.



Pilocarpine

The events associated with G protein-coupled receptor activation are as follows.

1. Agonist binds to the receptor, which has a high affinity for agonists at rest.
2. The binding of the agonist stabilizes a receptor conformation promotes receptor/ G protein coupling and allows GTP to exchange for GDP on the G protein α subunit.
3. The binding of GTP leads to the dissociation of the G protein from the receptor, thereby lowering agonist affinity. The agonist then dissociates from the activated receptor.
4. The G protein consists of three subunits (α, β, and γ) which also dissociate. The α subunit activates the appropriate second messenger system (e.g., phospholipase C for M_1 receptors). The β and γ subunits can exert independent actions.
5. The α subunit is inactivated by the hydrolysis of GTP to form GDP by a GTPase intrinsic to the G protein (GTPase activity may be activated by other intracellular proteins called GTPase activating

proteins [GAPs]).
6. The α subunit (with GDP bound) can then recombine with the β and γ subunits. The receptor is then in a high affinity state and ready for the binding of another agonist.

Alzheimer's disease

Alzheimer's disease is characterized by amyloid plaques and neurofibrillary tangles. Amyloid plaques contain deposits of β-amyloid, which is a 40-42 amino acid peptide derived from amyloid precursor protein. Neurofibrillary tangles contain a hyperphosphorylated τ protein, which forms paired helical filaments. Alzheimer's disease is associated with a loss of cholinergic neurons which project from the basal forebrain to the cerebral cortex and the hippocampus. The loss of cholinergic neurons is progressive and results in profound memory disturbances and irreversible impairment of cognitive function.

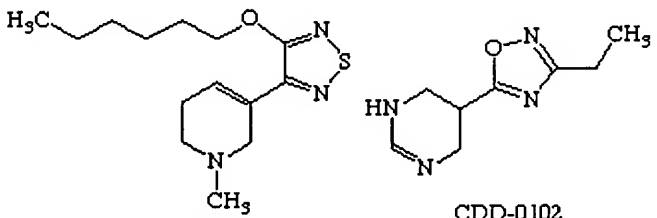
The cause of Alzheimer's disease is unknown, yet several genes and gene products (proteins) have been implicated.

- Mutations in APP (a small percentage of all Alzheimer's patients
- Presenillin mutations (may promote the formation of β-amyloid)
- Apolipoprotein E allele (E4 is associated with an increased risk of Alzheimer's disease)

Drug development

Recent efforts have focused on the development of centrally active muscarinic receptor agonists for the treatment of Alzheimer's disease. The rationale for therapy involves replacement of acetylcholine, which is depleted in Alzheimer's patients as the basal forebrain neurons degenerate. An ideal candidate for a drug would have several features including high CNS penetrance, high efficacy and selectivity for forebrain receptors and a low incidence of side effects.

The muscarinic agonist xanomeline is an arecoline derivative with very high affinity and selectivity for M1 muscarinic receptors. It contains a 1,2,5-thiadiazole ring, which is more stable than the ester found in arecoline. In CDD-0102 a 1,2,4-oxadiazole moiety serves as a suitable ester isostere.



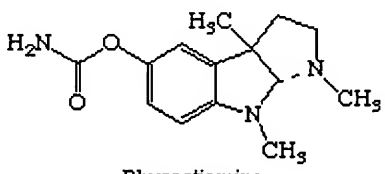
Acetylcholinesterase inhibitors

A variety of neurological and neuromuscular disorders involve a diminution of cholinergic activity.

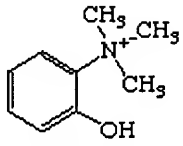
Often the most effective treatments are ligands which inhibit the breakdown of acetylcholine. Acetylcholinesterase inhibitors have been used clinically in the treatment of myasthenia gravis, a degenerative neuromuscular disorder, glaucoma and more recently Alzheimer's disease. In addition, cholinesterase inhibitors are widely utilized as pesticides and, if misused, can produce toxic responses in mammals and man.

Acetylcholinesterase is a tetrameric protein which catalyzes the hydrolysis of acetylcholine. The active site of AChEase includes a serine hydroxyl group, which is rendered more nucleophilic through the proton-acceptor action of a nearby histidine residue. The serine residue exerts a nucleophilic attack on the carbonyl carbon of ACh. A tetrahedral transition state is reached, which results in serine acetylation and the loss of free choline. The acetyl group binds to histidine as an N-acetate, but is hydrolyzed rapidly to yield free choline, acetate, and the free enzyme.

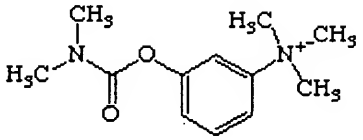
Some inhibitors of acetylcholinesterase act by competitively blocking hydrolysis, without reacting with the enzyme. Others inhibit by acylating the serine hydroxyl group, forming a carbamyl ester, which is more stable than acetate and less likely to leave the active site. The competitive blocker edrophonium is a quaternary compound that blocks the enzyme by binding to the active site.



Physostigmine



Edrophonium



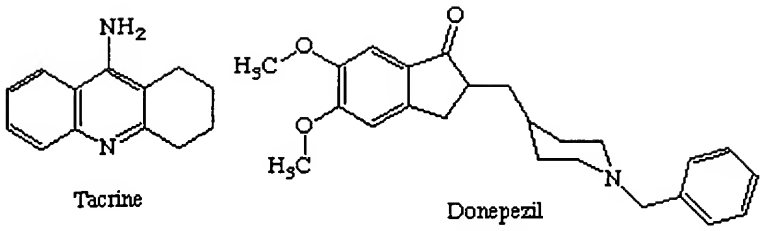
Neostigmine

The alkaloids physostigmine and neostigmine act as a metabolic inhibitors of acetylcholinesterase. The carbamyl ester formed by these compounds is much more stable than acetate (half-life measured in minutes as opposed to microseconds). The cholinesterase inhibitors are widely used to treat glaucoma (a disorder characterized by increased intraocular pressure). Acetylcholine reduces intraocular pressure, and cholinesterase inhibitors such as physostigmine are useful in treating the disease.

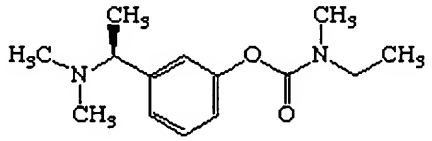
The other major use of cholinesterase inhibitors is for treatment of myasthenia gravis, an autoimmune disease in which antibodies are formed against the nicotinic receptor at the neuromuscular junction. The antibodies bind to nicotinic receptors to cause a profound muscle weakness and paralysis. Cholinesterase inhibitors can alleviate the symptoms of myasthenia by increasing muscle strength and endurance.

Recent efforts have been directed towards the development of novel strategies for the treatment of Alzheimer's disease. One strategy for the treatment of Alzheimer's patients has been the use of acetylcholinesterase inhibitors to increase the levels of acetylcholine in the synapse, thereby enhancing cholinergic activity in the affected brain regions. Physostigmine was used in early efforts to enhance cholinergic activity in the central nervous system although results were far from satisfactory.

Tetrahydroaminoacridine (THA, or tacrine) was the first cholinesterase inhibitor approved for use in Alzheimer's patients. Many patients given THA during clinical trials exhibited some alleviation of symptoms and some were able to resume normal activity and personal care. Not all patients respond to tacrine, and side effects include elevation of liver enzymes. Tetrahydroaminoacridine is formed from aminoacridine, an antimicrobial agent by hydrogenation of one of the rings. The resulting structure is no longer planar, and loses antibiotic activity, but does exert an action as a cholinesterase inhibitor.

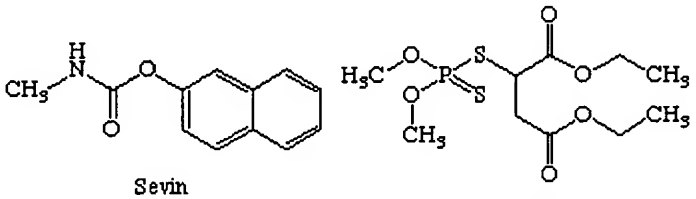


Within the past few years, other compounds have become available for clinical use. Donepezil is an cholinesterase inhibitor with improved selectivity for acetycholinesterase and good CNS penetration. It also exhibits lower toxicity than tacrine. Rivastigmine is another acetylcholinesterase inhibitor that has been approved for use in Europe.



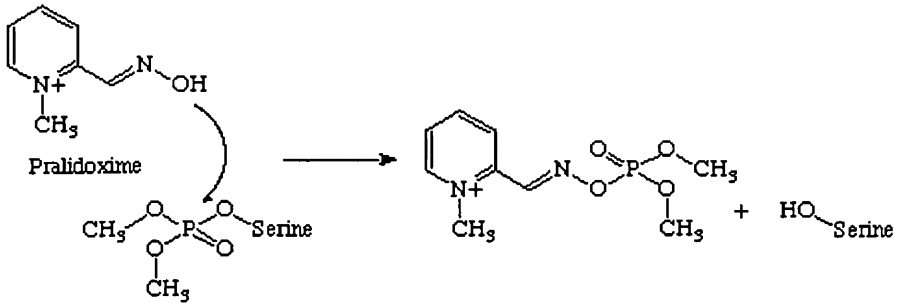
Rivastigmine

The insecticide carbaryl (Sevin), is uncharged and lipophilic and can penetrate the CNS of insects to act on the insect acetylcholinesterase, although the toxic effects on mammalian AChE are much lower. Malathion is another effective pesticide which is more effective on insects than on humans because it requires biotransformation to the phosphate form, which can only be carried out by insects.



Sevin Malathion

The molecule pralidoxime is a useful antidote for intoxication with cholinesterase inhibitors such as the organophosphates. The molecule removes the inhibitor from the active site in the form of an oxime phosphonate. Atropine also is used to block muscarinic responses due to excess acetylcholine. In addition, valium often is given as an antidote in conjunction with atropine to counteract seizures which may develop due to elevated levels of acetylcholine.



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This page was produced by Dr. William S. Messer, Jr., Professor of Medicinal and Biological Chemistry at The University of Toledo.